



1-T7  
1-7-00  
#15

PATENT  
ATTORNEY DOCKET NO. 07043/015007

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : M. Allen Northrup et al.  
Serial No.: 08/900,735  
Filed : 07/24/1997  
Title : MICROFABRICATED REACTOR

Art Unit: 1634  
Examiner: Sisson, B.

Assistant Commissioner for Patents  
Washington, DC 20231

DECLARATION UNDER 37 CFR § 1.131

We, M. Allen Northrup and Richard M. White, declare as follows:

1. We are the coinventors of the invention described in the claims of the above-identified patent application, as amended by the Response filed on July 12, 1999. This Declaration is a replacement for the Declaration filed in the Patent and Trademark Office on July 12, 1999.

2. Prior to May 1, 1992, we completed the conception of the invention in this country as evidenced by the following:

- Prior to May 1, 1992, we prepared and signed an Invention Disclosure Statement entitled "Microinstrumentation-based Polymerase Chain Reaction (PCR) Diagnostics" (Exhibit A). The Invention Disclosure Statement describes the features of an apparatus ("the apparatus") for amplifying a preselected polynucleotide in a sample. This apparatus included a reaction chamber and at least one reactant chamber, at least one channel interconnecting the reaction and reactant chambers, a heater configured to heat reactants in the reaction chamber, a temperature controller coupled to the heater and configured to control the temperature of a reaction in the reaction chamber, and a product analysis chamber coupled to the reaction chamber and adapted to analyze reaction products contained in the product analysis chamber, as recited in independent claims 1 and 104 of the above-identified application.

3. Prior to May 1, 1992, in this country, we reduced to practice various features of the apparatus recited in independent claims 1 and 104, as evidenced by the following:

- In a notebook kept by M. Allen Northrup ("Dr. Northrup"), an entry (Exhibit B) dated before May 1, 1992, contains a description of an apparatus ("apparatus A") that embodies some of the features of the apparatus recited in independent claims 1 and 104. For convenience, Exhibit B has been recently annotated in red ink, with the various features of the apparatus labeled according to their respective reference numbers in FIGS. 2 and 3 of the above-identified patent application. In particular, apparatus A comprises:

- a reaction chamber configured to contain a chemical reaction (see, e.g., 30);
  - a heater configured to heat reactants in the reaction chamber (see, e.g., "polyheater/ polysilicon" 54b); and

- a temperature controller coupled to the heater and configured to control the temperature of a reaction in the reaction chamber (see, e.g., 56br, 67br, 56bl and 67bl).

- In the above-mentioned notebook kept by Dr. Northrup, an entry (Exhibit C) dated before May 1, 1992, describes the results of certain operational tests on apparatus A, constructed (see, e.g., photographs on page 34) and tested prior to May 1, 1992.

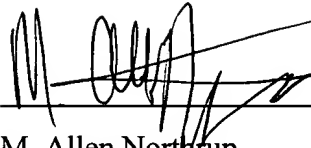
- In the above-mentioned notebook kept by Dr. Northrup, an entry (Exhibit D) dated before May 1, 1992, describes the results of certain operational tests on apparatus A. In particular, apparatus A was successfully operated to amplify a preselected nucleotide (see, e.g., photographs on page 45).

- In the above-mentioned notebook kept by Dr. Northrup, an entry (Exhibit E) dated before May 1, 1992, describes the results of certain operational tests on apparatus A. In particular, the tests included homogeneous detection of the reaction products in the reaction chamber using UV light.

4. Each of the dates deleted from Exhibits A-E is prior to May 1, 1992.

5. We hereby declare that all statements made of our own knowledge are true and that all statements made on information and belief are believed to be true. We understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the application or any patent issuing thereon.

Date: Dec 20 1999



M. Allen Northrup

Date: 17 Dec. 1999 Richard M. White  
Richard M. White



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Assistant Commissioner for Patents  
Washington, DC 20231

EXHIBITS FOR DECLARATION OF  
M. ALLEN NORTHRUP AND RICHARD M. WHITE

Date of Deposit January 5, 2000  
I hereby certify under 37 CFR 1.8(a) that this correspondence is  
being deposited with the United States Postal Service as **first class**  
mail with sufficient postage on the date indicated above and is  
addressed to the Assistant Commissioner for Patents, Washington,  
D.C. 20231.

Bridget Conger  
BRIDGET CONGR

A

## Invention Disclosure Statement

Title: Microinstrumentation-based Polymerase Chain Reaction (PCR) Diagnostics

Inventors:

M. Allen Northrup  
923 Creston Rd  
Berkeley, CA 94708

Richard M. White  
350 Panoramic Rd  
Berkeley, CA 94708

Date:

### Background:

91  
The polymerase chain reaction (PCR) is a method by which a single molecule of DNA (or RNA) of an organism can be selectively amplified several millionfold within a few hours. This well-established procedure is based on the repetition of heating (denaturing) and cooling (annealing) cycles in the presence of the original DNA molecule, specific DNA primers, dNTPS, and DNA polymerase enzymes. Each cycle produces a doubling of the target DNA segment, leading to an exponential accumulation of the target segment. The generalized procedure involves: 1) processing of the sample to release target DNA molecules into a crude extract, 2) addition of an aqueous solution containing enzymes, buffers, deoxyribonucleotide triphosphates (dNTPS), and two oligonucleotide primers, 3) thermal cycling of the reaction mixture at two or three temperatures (i.e., 94, 72, and 37-54 °C) for typically 20 to 30 cycles, and 4) amplified DNA detection. Intermediate steps are introduced in some assays to incorporate signal-producing and/or surface-binding primers, and to purify the reaction products (e.g., electrophoresis or chromatography). Reaction volumes and times are typically on the order of tens of  $\mu$ Ls and one to two hours, respectively. PCR-based technology has been applied to a variety of analyses, including environmental and industrial contaminant identification, medical diagnostics, and biological research.

92  
Monolithic microfabrication technology has advanced to the point where a variety of micro-scale components can be made that have electrical, mechanical, optical, chemical, and thermal capabilities. For example, devices have been fabricated that can pump, heat, cool, and mix microliter quantities of solids and liquids. As well, micro-scale optical and electromechanical/chemical physical and chemical sensors have been developed such as fiber optic probes and Lamb-wave sensors. The integration of these devices into systems allows the development of analytical instruments on a micro-scale. The advantages of such integrated devices include the ability to manufacture them in batch quantities with high precision, yet low cost. Their inherent small size also provides significant advantage in that they would be able to perform highly automated *in situ* analyses.

### Invention Concept

93  
The invention disclosure herein concerns the application of microinstrumentation to PCR. The small analytical and reaction volumes of PCR make it an ideal diagnostic technique for



implementation on micro-devices. Such a system could contain reservoirs of reagents, agitation and mixing devices to process the target cells, pumps to carry solid and/or fluid reagents to mixing chambers, heaters and coolers to perform the denaturing and annealing cycles, optical and/or electromechanical/chemical sensors to discriminate the reagents and products of the reaction, and separation devices to purify reactants and products. Feedback control via integrated sensors could also be incorporated directly into the system.

Many or all of these devices could be made from microfabrication technology and could process micro- to picoliter volumes. By the selection and integration of appropriate microfabricated devices, a precise and reliable reaction and analysis instrument for PCR-based diagnostics could be devised. A schematic diagram of an example of one such possible system is presented in Figure 1. Several to many of these micro-instruments could be manufactured on a wafer and could run in parallel, allowing the processing and analysis of several target agents and controls. Target DNA detection methodology could include either an optical, electromechanical, electrochemical, or a combination sensing device. Detection signals could be processed and stored with microelectronic devices.

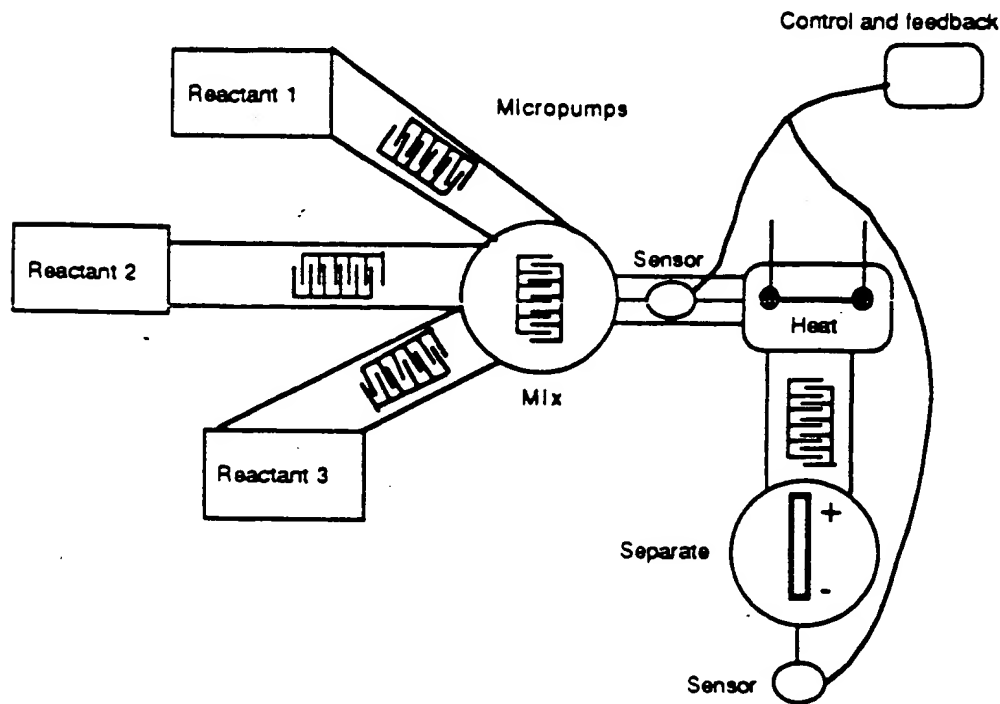
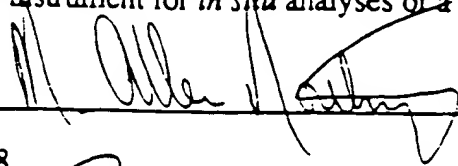


Figure 1. An example of an integrated microinstrument.

In summary, in this disclosure we describe an integrated microsystem and analytical instrument to perform PCR-based diagnostic methodology. The amplification process from minute sample sizes and reaction volumes, and specific reaction sequence of the PCR technique plays favorably into the micro-device capabilities of on-going microfabrication technology. The development of this integrated micro-PCR system will lead to a highly automated, miniaturized, analytical instrument for *in situ* analyses of a variety of samples.


Inventors:

M. Allen Northrup  
923 Creston Rd  
Berkeley, CA 94708



Date \_\_\_\_\_

Richard M. White  
350 Panoramic Rd  
Berkeley, CA 94708



Date \_\_\_\_\_

Witnesses:



Date \_\_\_\_\_



Date \_\_\_\_\_

CLASS CODE

(SEE INSTRUCTIONS ON REVERSE SIDE)

COMPLETE ITEMS 1-4 - USE ADDITIONAL SHEETS AS NECESSARY

1. TITLE OF INVENTION

MICROINSTRUMENTATION-BASED POLYMERASE CHAIN  
REACTION (PCR) DIAGNOSTICS

2. INVENTOR(S)

TITLE

CAMPUS UNIT OR MAILING ADDRESS

M. ALLEN NORTHROP  
RICHARD M. WHITE

POST DOCTORAL  
FELLOW (LLNL);  
VISITING SCHOLAR  
(UCB)  
PROFESSOR

LLNL; BSAC  
↑  
BSAC; EECs DEPT.

3. CONTRACT OR GRANT NO.(S)

SPONSOR(S)

PRINCIPAL INVESTIGATOR

N/A

-

-

4. EVENTS

DATE

REFERENCES & COMMENTS

A. Initial Idea

TELEPHONE CONVER-  
SATION BETWEEN THE

B. First description of complete invention, oral or written  
(conception)\*

INVENTORS  
THIS DISCLOSURE  
ATTACHED

C. First successful demonstration, if any (first actual reduc-  
tion to practice)\*

NOT YET

D. First publication containing full description of invention  
(establishment of publication bar)\*

NOT YET

E. External oral disclosures

NONE

5. BRIEF ABSTRACT OF INVENTION - ATTACH DETAILED DESCRIPTION

INVENTION CONCERNS APPLICATION OF MICRO-  
STRUCTURES TO INSTRUMENT PCR AMPLIFICATION OF  
DNA, ETC. A SPECIFIC EMBODIMENT EMPLOYING THE  
LAMB-WAVE ULTRASONIC TECHNOLOGY IS SHOWN.

KEYWORDS (OTL USE ONLY)

6. INVENTION SUBMITTED BY:

INVENTION DISCLOSED AND UNDERSTOOD BY:

MAN

Inventor's Signature

Date

MAN

Co-Inventor's Signature

Date

Co-Inventor's Signature

Date

Co-Inventor's Signature

Date

Witness' Signature

Date

Print Name

Reviewed by:

Licensing Associate

Date

\* See instructions on back.  
- Please have PI sign if PI is not an inventor

UNIVERSITY OF CALIFORNIA , BERKELEY (UCB)  
OFFICE OF TECHNOLOGY LICENSING



AGREEMENT CONCERNING DEVELOPMENT OF TECHNOLOGY  
AND DISTRIBUTION OF INCOME

Case No B \_\_\_\_\_

Name of Technology: Microinstrumentation-Based Polymerase Chain Reaction (PCR)  
Diagnostics

Creators: M. Allen Northrup and Richard M. White

Reference: University of California Patent Policy as revised.

- 1 UCB and Creator(s) desire that the above Technology be licensed by UCB to industry in order that applications and uses of the Technology be made widely available for public use and benefit. Creator therefore assigns to UCB any right, title, and interest he or she may have in the Technology including, but not limited to, patent, copyright, tangible research materials, and semiconductor mask work rights, and assures UCB that he or she has not granted any such rights in Technology to any other person or entity. The term "tangible research materials" refers to research results which are in tangible form as distinct from intangible (or intellectual) property. Examples include integrated circuit chips, computer software, biological organisms, engineering prototypes, engineering drawings and other property which can be physically distributed.
- 2 UCB shall take such actions as it believes appropriate to make the Technology available for public use and benefit, but shall not be liable for any failure to generate income thereby.
- 3 Creator agrees to cooperate with UCB to secure and protect UCB's interest and ownership in the Technology, including executing patent assignment and other pertinent documents, giving testimony, and providing pertinent information; provided, however, that if any expenses are incurred by Creators in providing such cooperation, such expenses shall be paid by UCB.
- 4 Considering the foregoing, Net Royalty Income will be distributed as follows:

**CREATOR(S) SHARE:** 33 1/3% of Net Royalties  
**DEPARTMENT SHARE:** 50% of Adjusted Net Royalties  
**UNIVERSITY SHARE:** 50% of Adjusted Net Royalties

The academic department(s) (or organized research unit(s)) of the creators are:

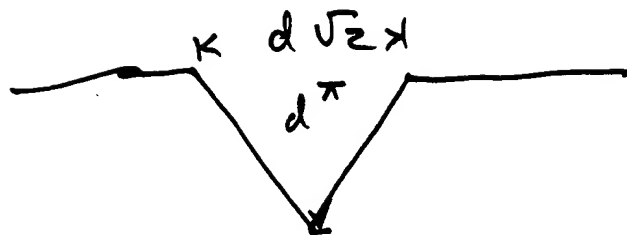
Electrical Engineering and Computer Sciences Department and  
Berkeley Sensor and Actuator Center

- 5 "Net royalties" are defined as gross royalties and fees, less 15% thereof for administrative costs, and less the out-of-pocket costs of patenting, protecting, and preserving patent rights, maintaining patents, the licensing of patent and related property rights, and such other costs, taxes, or reimbursements as may be necessary or required by law, and a reserve to cover out-of-pocket expenses which UCB reasonably determines may be incurred in following fiscal years which may not be covered by future royalty revenue. When no longer needed, UCB agrees to distribute the balance of funds reserved according to the formula of paragraph 4 above.
- 6 "Adjusted Net Royalties" are defined as "Net Royalties," as specified in Paragraph 5 above, less the following deductions to such Net Royalties thereby calculated:

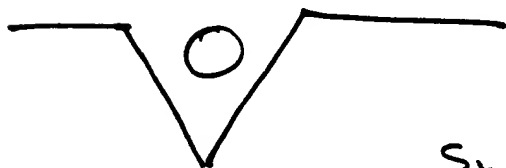
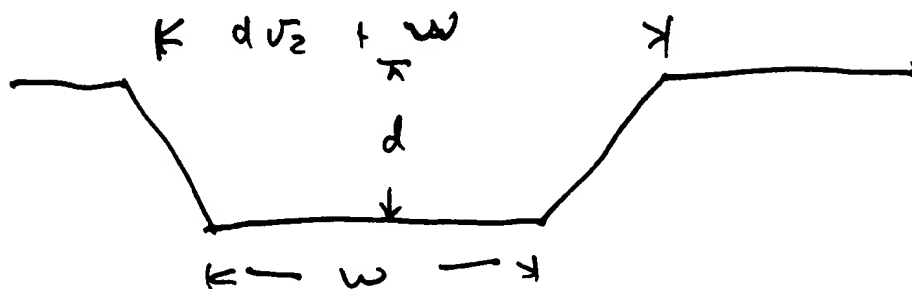
OTL #2

Rev.

B



Scribe line  $\approx$  70% thickness



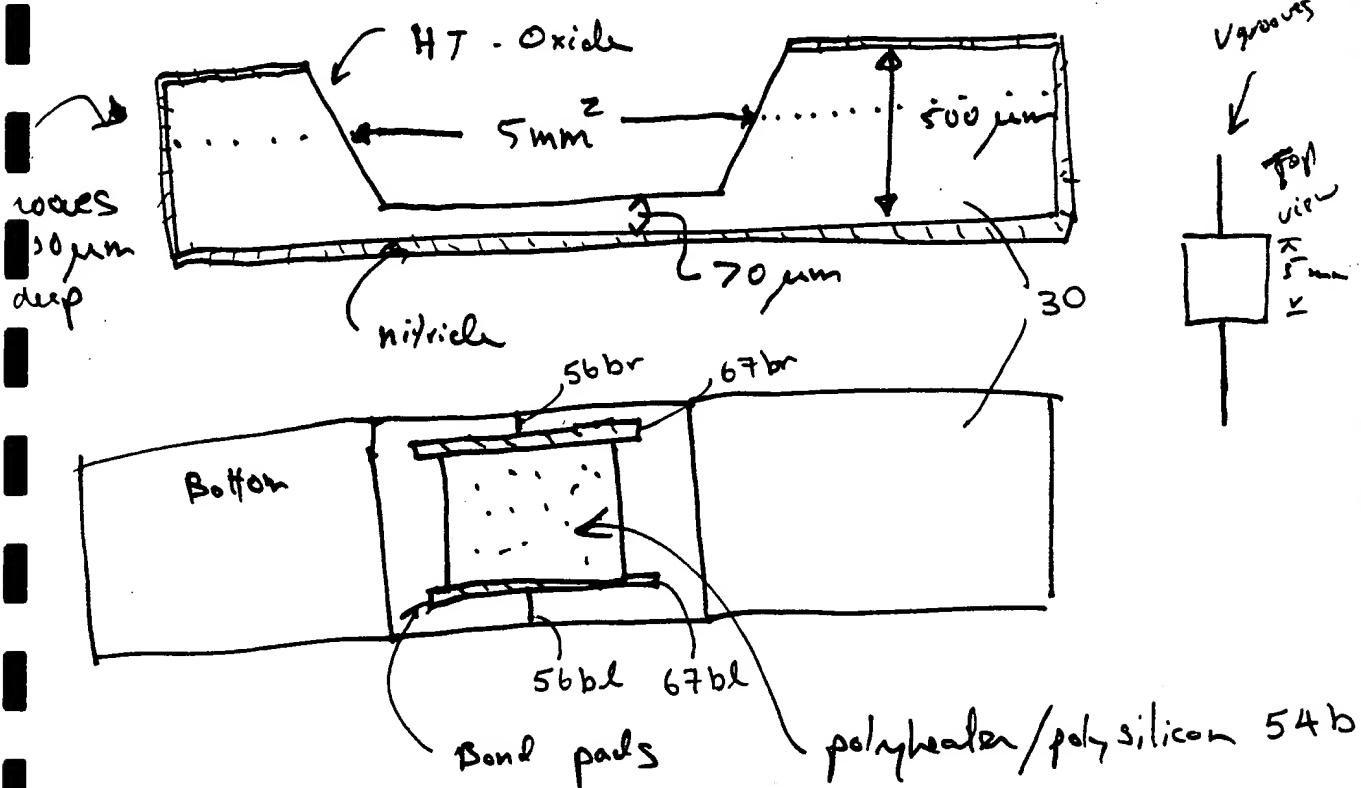
or

Syringe - U. groove



M. All

B) Summary of recent design meeting with Courtney and Jim on



Processing Steps:

- 1) RCA Clean
- 2) Tunnel oxide
- 3) PolySilicon dep.
- 4) Dope poly (phos) dep. here
- 5) dep. Si<sub>3</sub>N<sub>4</sub>
- 6) Duffman well-side
  - 1) wells
  - 2) V-grooves
  - 3) Scribe lines
- 7) Plasma etch
- 8) KOH - timed etch
- 9) Strip Si<sub>3</sub>N<sub>4</sub>
- 10) Strip oxide

C



M. All Day

© Cetus w/ Mita Ching

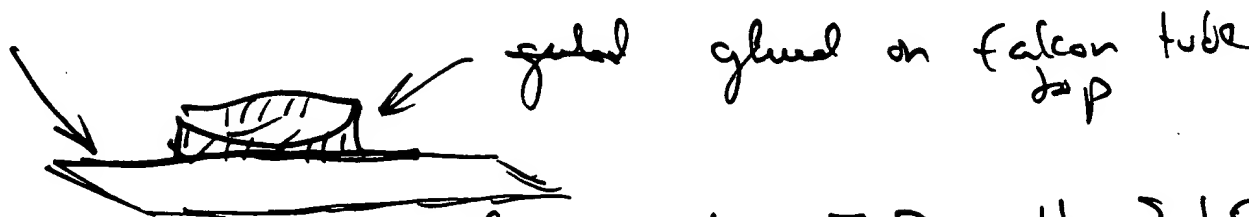
PCR rxns on miles heaters 35 ~~heaters~~ heaters  
Volume ~ 25  $\mu$ l volume wells

1) Standard rxns 20, 20, 30, 40  $\mu$ l

20, 30, 40  $\mu$ l w/ graphite pencil tips  
~ 60  $\mu$ l oil in each

Wgth Temp:  
max = 45°C  
min = 42°C

2) Device: ~ 60  $\mu$ l rxn mixture  
~ excess excess oil



Cycling w/ 7.7 volts } 1.5 W  
200 mA

oil warms up {  
upline ~ 47 sec / 29 sec = 21<sup>st</sup> cycle  
down = 23 sec / 15 sec = " "

- Thermocouple touching membrane center  
- Type (?)  
- Diameter .005" = 100  $\mu$ m  
tip ~ 200  $\mu$ m

poly = ~~0.6~~ thickness 0.6  $\mu$ m  
clonin: 0.3  $\mu$ m

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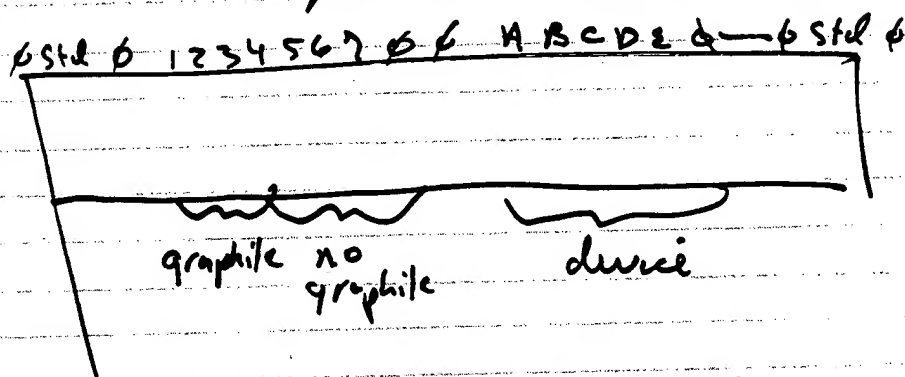
Cont

M. Allroy

- 30 cycles completed on dice

- 25 cycles on standards

Gel:

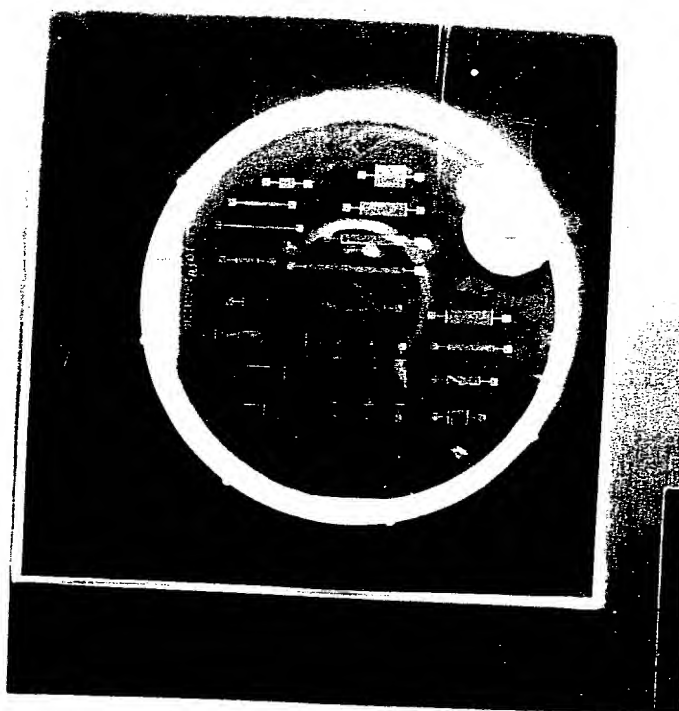
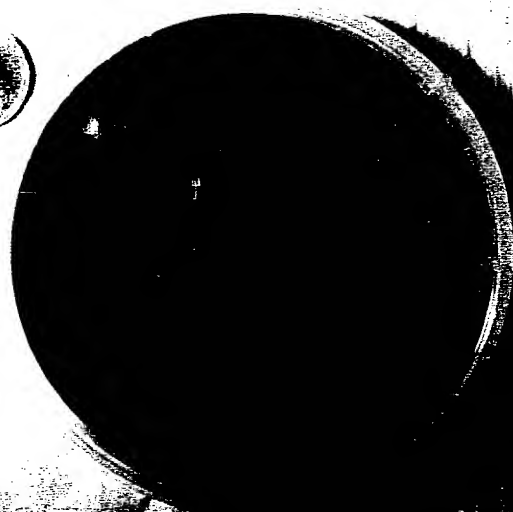


1	20	nl	w/ graphite
2	30		
3	40		
4	20	nl	w/out graphite
5	20		
6	20		
7	40		

25  
cycles

A-E = dice = 30 cycles

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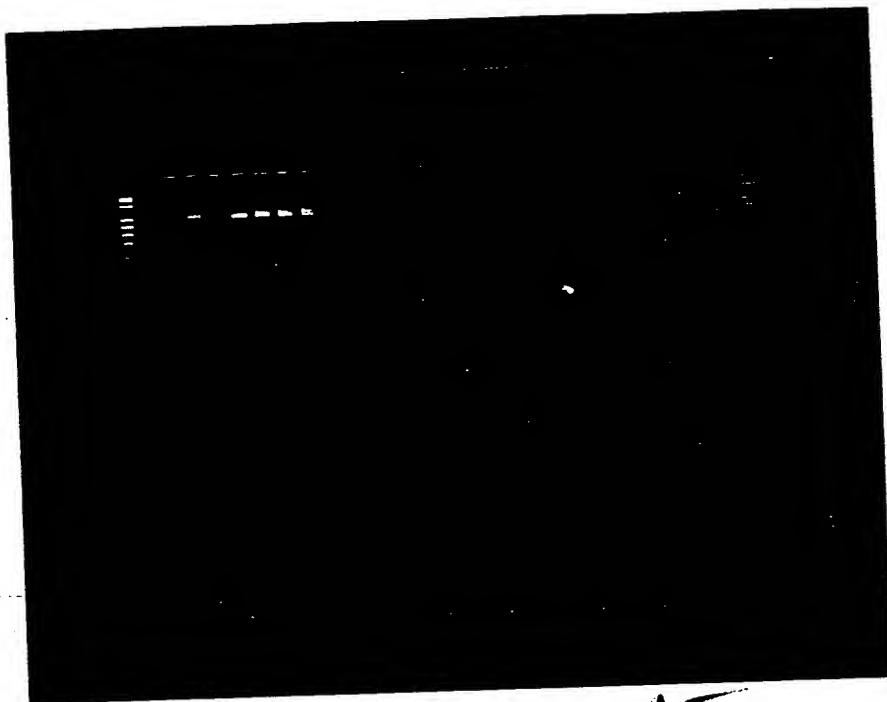


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M. All *any*

35

4423  
X1  
F. 4.5



M. All *any*

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### Results:

- Graphite did not have a significant effect (lanes 1-3)
- Primer Dimer formed in wells due lanes (9-11) probably to not reaching high enough T for lambda to denature
- note this system has 2-bare overlap which is least toward primer-dimer formation
- evidence of steep T-gradient
- Try high T (40°) longer 1 min.

D

taped receipt from  
Watson

✓

Run	50 pl 10x Rf	2
pa		
B. Watson	50 pl 1mm d/TP	3
	50 pl W13	4
	10 pl 10x10 = 100 patches	5
	photo	
M. Allen	10 pl photo	6
	2 1/2 pl 10x1.750/PCR	12.5
	12.5/50/pl = 2.5	7
	327.5 W20	
	<hr/>	
	500	

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Cellus M. All ~~page~~

Try new PCR system  
(more Temp forgiving)

142 bp product target as SS M13 from  
gag-region of HIV

1) Starting target =  $10^8$  copies in 5  $\mu$ l

T = 96-55

(works at 88+)

16-18 cycles  
is plenty

2) primers

old names:

SK145

SK431

new names

ph07

ph08

10  $\mu$ l/ml

Reaction mixture: (500  $\mu$ l)

50  $\mu$ l 10x Buffer w/ MgCl

" 1 mM dNTPs

" M13 w/ gag region of HIV

10  $\mu$ l =  $10^8 \times 10 = 100$  pmoles

10  $\mu$ l (same for) ph07  
ph08

?

2 1/2  $\mu$ l = 10  $\times$  1.25  $\mu$ l/pul 12.5  
Tag

327.5  $\Delta$ H<sub>2</sub>O

500  $\mu$ l total rxn volume

100.0  
172.12  
327.5

M. All *[signature]*

(cont)

- 1) re-use voltage (same device) as  
on mach 30 (ie 3.17 V ~ 98°C)  
at 0.2A

Do only 20 cyclesA) Standards

10, 10, 20, 20, 30, 30, 40, 40

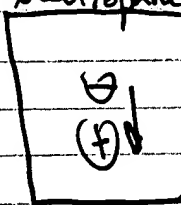
w/ 50  $\mu$ l oil (1-8)

- B) Device 30  $\mu$ l w ~ 90  $\mu$ l oil

1-minute cycles at 3.17V  
20-1 minute cycles (A-E) 0.2A

electrophoresis

well-problem



p std 6 1 2 3 4 5 6 7 8 9 10 A B C D E F 4146

wire connectors

- 1a) Had to re-solder device  $\nabla$  after 2-cycles  
fix time  $\approx$  1/2 hour rxn was  
at room temp

Results - ① formed product in both  
stds and in wells  
② wells (and 1) std had  
less bright primer-dimers  
③ device provided ~6-5  $\mu$ l gel

See next  
2 pages:

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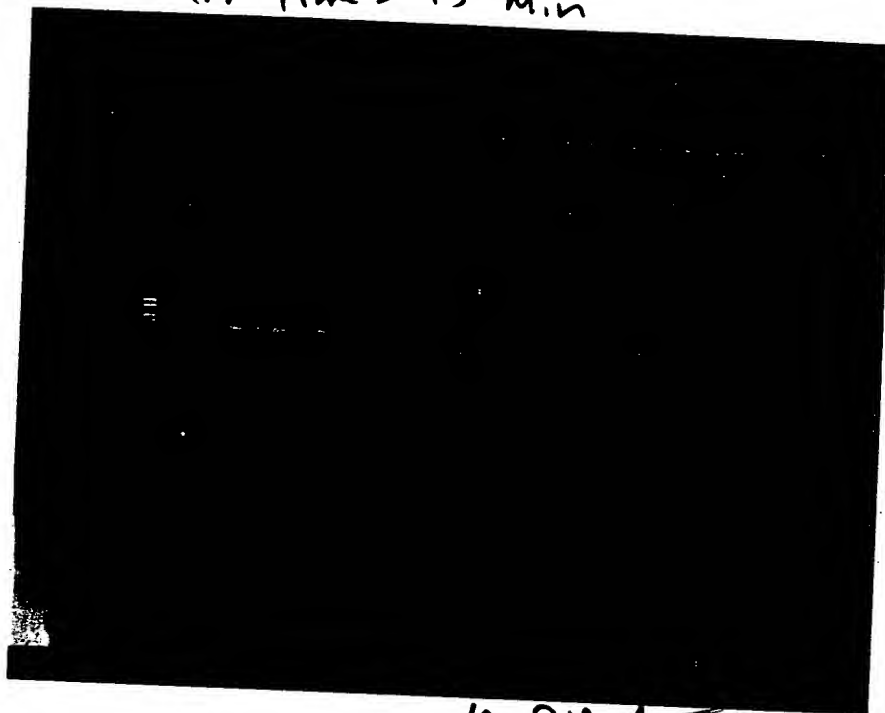


44

Cont results (photos)

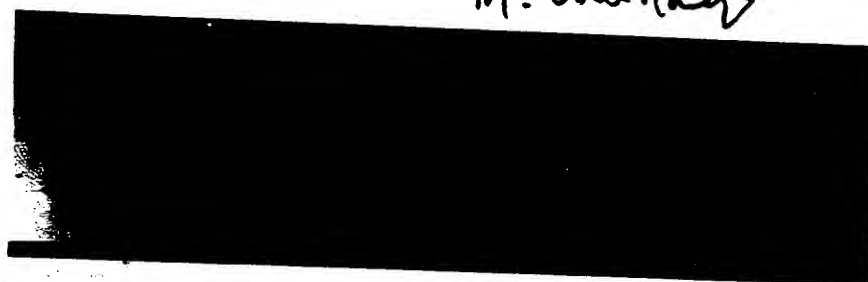
M. Allen

electr. Time = 15 min



0225 9.4 3200 T=1 sec

M. Allen



101

T=1 sec 9.4 3200

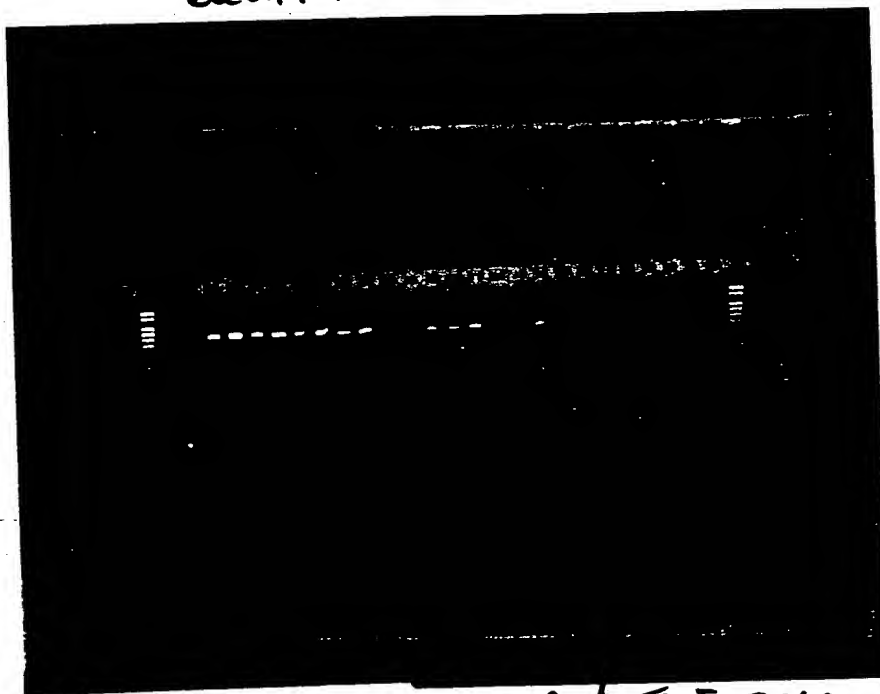
#1 loaned to Mike Ching

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cont  
Results (photos)  
Devia PCE results positive

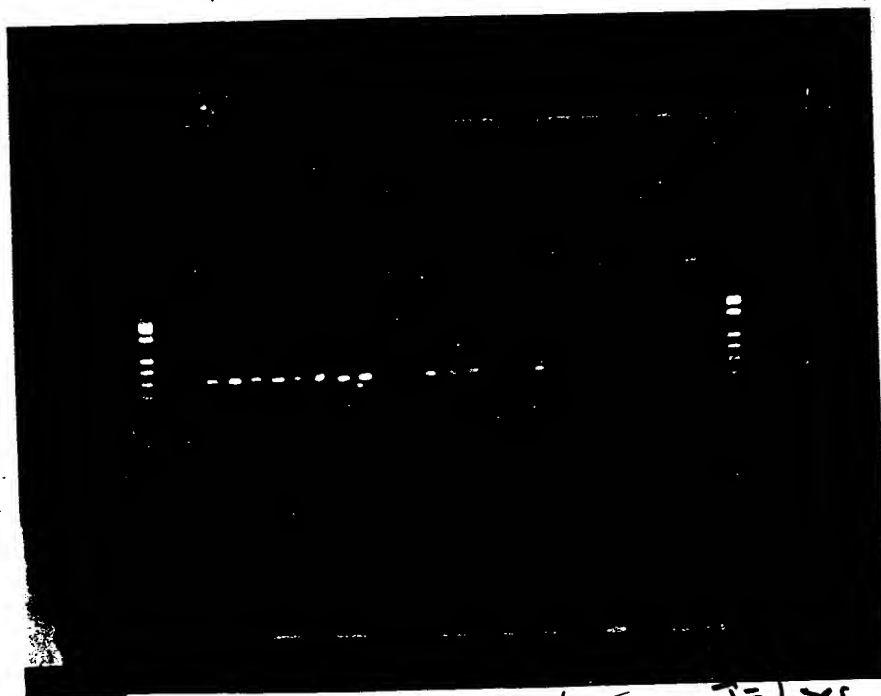
M. All <sup>45</sup>

elec. T = 15 min



M. All <sup>T = 24C</sup>  
4.6 7200

elec T = 40 min



M. All <sup>T = 24C</sup>  
4.6 7200

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Cont  
Results

M. Allaway

47

Notes (Signal back of several (not all)  
results photos with this pen (other (on front)  
was ~~not~~ permanent ink)

- PCR (HIV - MSP) worked well in  
integrated-heater devices, gel electrophoresis  
verified product. Some, but minimal Primase  
(esp. due to known fact that device  
reaction mixture cycled 1-2 times,  
then at R.T. for  $\frac{1}{2}$  hr & prior to  
20 cycles due to need to re-solder  
connections - new rxn mixture (30  $\mu$ l)  
was added)

- was able to extract ~100% of aqueous  
phase with 200  $\mu$ l (set at 30  $\mu$ l)  
pipette & load 5-6 wells of  
electrophoresis channel

(\*)  $\rightarrow$  calculate power consumed in today's  
experiment compare to batteries

### Other Discussion

Last Tues w/ Ray Manilla  
here (Cetus) along w/  
Russ Higuchi, Bob Watson, Russ's  
technician, myself we tried  
homogeneous detection w/ video  
CCD over 460 thermal cycles

- pulsed  $\text{He}$ -laser (ILEE laser  
company, Switz) was tried

$\rightarrow$  see LLNL Book (notebook)  
for details

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E

M. Allington

New Device

17  $\Omega$ 4.50 V  $\approx 98^\circ\text{C} \pm 2(?)$ 

0.20 A

a)

Bridge

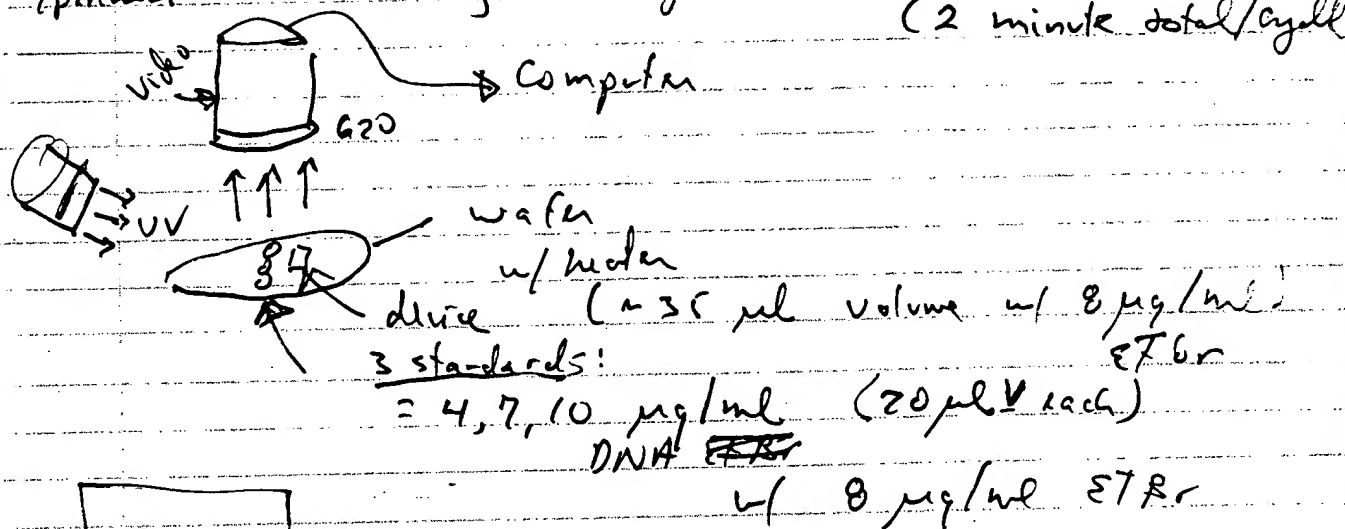
Homogeneous  
direction  
w/ UV  
lightb) 2nd device: 37  $\Omega$ 5.89 V 0.17 A  $\approx 98^\circ\text{C}$ 

Experiment

Try 20 cycles

1 minute each

(2 minute total/cycle)



0:1 ran
out

Try again!

reload w/ same rxn mixture

rxn = 25  $\mu\text{l}$  on device

Homogeneous did not work

- note standards weren't even  
consistent- try w/ new batch of .4 x .4 cm<sup>2</sup>  
hecter wells